

Biological Control Strategies for *Culex pipiens*

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Abstract

Background: The mosquito species *Culex pipiens* is known to carry serious diseases like West Nile virus and lymphatic filariasis. Traditional methods that rely on chemicals to control their populations can be harmful to the environment and human health, which is why researchers are exploring more natural, biological options.

Main Text: This study investigates different biological control methods, including microbiological agents, natural predators, and plant-based treatments, with a particular focus on *Bacillus thuringiensis israelensis* (BTI). The key finding is that BTI is the most effective biological larvicide because it is highly specific, safe for other organisms, and works by targeting *Culex pipiens* larvae with toxins called Cry and Cyt. These toxins damage the larvae's gut membranes, leading to septicaemia (blood poisoning). Other promising methods include utilising *Wolbachia* bacteria, which reduce mosquito fertility, and fungi like *Beauveria bassiana* that infect and kill larvae. Besides, some larger organisms like larvivorous fish (*Danio rerio*) and certain plant extracts (*Teucrium polium*) have shown potential as larvicides. However, challenges such as temperature sensitivity, slower rates of action, and mosquitoes developing resistance are important considerations. The study emphasises the importance of combining different methods, like using both BTI and *Lysinibacillus sphaericus*, to improve effectiveness and reduce the chance of resistance developing.

Conclusion: In summary, biological control provides a sustainable and environmentally friendly alternative to chemical pesticides for managing *Culex pipiens*. Among these options, BTI stands out because of its targeted approach and additional benefits, such as breaking down pollutants. Moving forward, research should focus on genetic modifications to improve these biological agents' specificity and resistance management, aiming for effective control that also maintains healthy ecosystems.

Keywords: *Culex pipiens*, Vector control, *Bacillus thuringiensis*, Microbial larvicides, Resistance management

Background

Culex pipiens is a common mosquito species that transmits various illnesses, including West Nile virus (in temperate regions) and lymphatic filariasis (in tropical regions). This study explores biological control techniques for *Culex pipiens*, focusing on microbiological agents, natural predators, and plant-based treatments. *Bacillus thuringiensis israelensis* (BTI) is the most popular biological larvicide due to its effectiveness, host specificity, and environmental safety. The review discusses the molecular processes of BTI's Cry and Cyt toxins, compares BTI with alternative approaches, and discusses resistance development issues. Limitations of biological control include temperature dependency and slower effects.

A narrative review of literature obtained using the keywords to search from the Scholar Google database through the Publish or Perish software. The first one hundred literature were evaluated.

Main Text

General characteristics of *Culex pipiens*

Culex pipiens is a bloodsucking arthropod that belongs to the order Diptera. It is also one of the most prevalent varieties of mosquitoes, renowned for its attraction for people and animals, since it prefers areas with large concentrations of organic debris. *Culex pipiens* (Diptera: Culicidae) is worldwide common mosquito insect worldwide in many areas (1). Mosquito-borne illnesses pose a significant worldwide health issue (2). The matter is not limited to one pathogenic organism, but rather

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this small insect can transmit several pathogens at the same time and this mosquito carrying pathogens transmits diseases whose symptoms may be simple, such as fever, and whether severe or not, some of these symptoms develop and lead to the death of the host (3, 4). Such pathogens include viruses (e.g., *Dengue virus*, *yellow fever virus*, *Japanese Encephalitis Virus*, *Chikungunya virus* and *Zika virus*) and protozoa (e.g., *Plasmodium falciparum*) (3, 4).

The spread of the threat of these easily transmitted pathogens was and is still a threat to the lives of many and the global economy (5). Although these pathogens are more likely to be transmitted by mosquitoes, the distinctive appearance of some types of pathogenic bacteria is not hidden (e.g., *Staphylococcus*, *Streptococcus*, *Xanthomonas*, *Sphingomonas*, *Shigella*, *Salmonella* and *Klebsiella* and many other species) (6). Furthermore, Dengue fever has grown thirtyfold in the previous 50 years around the world (7). Over 100 nations have declared an active outbreak of the Chikungunya virus (CHIKV) by 2023, posing a threat to approximately 1.5 billion people (8). Mosquito-borne illnesses can be reduced by using chemicals that remove any presence of mosquitoes, meaning their effects on humans, or removing places where mosquitoes colonise, may be considered a suboptimal method, but useful. Either way, the ideal method is using materials or organisms or their extracts to control the presence of mosquitoes (9).

Mosquito distribution and classification

Mosquitoes are found throughout the world, particularly in tropical and subtropical locations

where the climate is warm and humid, making breeding conditions ideal. *Culex pipiens* mosquito larvae are detected in millilitres of water. Each millilitre of water may hold an egg boat with hundreds of eggs. It is mostly found in sub-Saharan Africa, Southeast Asia, and Latin America, with a few species being found in the Middle East and northern Australia. *Culex pipiens* Linnaeus, 1758, is a recognised vector of WNV. This species is endemic, widely distributed, and prolific throughout Europe (10). *Culex pipiens* mosquitoes are insects that belong to the order Diptera and the family Culicidae (11). The Culicidae family contains three subfamilies: Toxorhynchitinae, Anophelinae, and Culicinae. Toxorhynchitinae has only one species, *Toxorhynchites*, and is of minimal medicinal significance because it feeds on nectar rather than blood (12). Culicidae consists of 41 genera and around 3,500 species (13, 14). The species of *Culex pipiens* has two bioforms: *Culex pipiens pipiens* and *Culex pipiens molestus* (15). This mosquito undergoes complete metamorphosis, passing through four stages (egg, larva, pupa, and adult) as shown in Figure 1. The mosquito's nutrition varies during these stages, as it feeds on organic materials in its aquatic environment until it reaches the adult stage, in which its nutrition varies from the female to the male. While the male feeds on sugary materials, the female feeds on the blood of its host. Understanding the mosquito's life cycle and identifying the targeted stage helps in understanding the mechanism of action of pesticides that are to be developed to accurately target this insect.

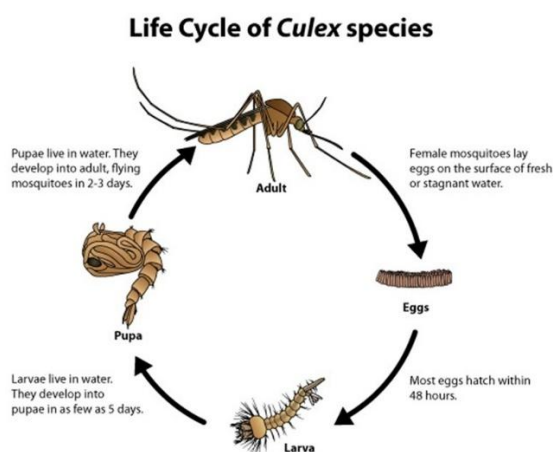


Figure 1: Life cycle of *Culex pipiens*

Biological control strategies

Using microscopic or enormously creatures to eliminate mosquito larvae from their surroundings is the greenest method (16). At first, the use of chemicals was the ideal solution when

the ability of mosquitoes to transmit diseases was confirmed, but the harms of using such materials were not hidden from everyone, without exception, including soil, water, and wildlife (17). The effects of these substances have been

harmful to children, poisoning them and even causing allergies and damaging their respiratory system, which leads to death in several countries (18). Biological control, also known as classical or importation biological control, is an environmentally friendly strategy used to manage harmful insect populations by introducing their natural predators, parasites, or pathogens. The goal is to restore ecological balance and reduce the need for chemical pesticides, which can harm non-target species and the environment (19). Who would think that a small insect might develop resistance to these chemicals, which has led to the re-emergence and invasion of these pests in human settlements? Spraying pesticides was not successful and only caused environmental pollution and killed non-target organisms (20). The emergence of the role of plant extracts and fungal extracts is not hidden by using bacterial species. Such compounds that are less toxic or vulnerable to chemical breakdown, viruses, larvivorous fish, toxorhynchites larvae and nematodes and microorganisms, as well as different bacterial species (21). The organisms utilised in this sort of management differ in size and effect, necessitating categorisation into two major groups: macroorganisms and microorganisms, depending on their properties and roles in the ecosystem.

Microorganisms

Bacterium (e.g., *Bacillus thuringiensis*), viruses (e.g., *Nucleopolyhedrovirus*, *Cyprovirus*) that infect and destroy pests and fungi such as *Beauveria bassiana* and *Trichoderma harzianum*.

Bacterial Biocontrol

Wolbachia bacteria have been identified as a potential strategy for controlling mosquito-borne illnesses through reproductive manipulation and genetic male conversion (22). *Wolbachia*, a biotechnology promising in managing vector-borne diseases, has earned public trust in 13 countries. However, complex ecological interactions require multidisciplinary approaches for long-term control. Understanding *Wolbachia*'s genetic mechanisms could lead to targeted genetic approaches (23). It is a safe and successful biotechnology with public support in over thirteen nations. It can limit human pathogen spread in *Culex* populations, but a multidisciplinary strategy is needed for effective, long-term, and sustainable regulation of complex ecological interactions (23). The other bacterial in nature companions [*Xenorhabdus* and *Photorhabdus* spp. (Enterobacterales: Morganellaceae)] regarding entomopathogenic parasites (EPNs) (*Sterinernema* spp. and

Heterorhabditis spp.) (Rhabditida: Heterorhabditidae and Steinernematidae)] they are one of the green techniques to eliminate a range of insect-borne diseases (24).

Viral biocontrol

Mosquitoes are infected with various viral pathogens, primarily categorised into four major groups: baculoviruses (NPVs), cytoplasmic polyhedrosis viruses (CPVs), densovirus (DNVs), and iridoviruses (MIVs). These DNA viruses cause disease, while cypoviruses are the main RNA viruses (25). Nucleopolyhedra viruses (NPV) are being researched as a safe and efficient biological pesticide. A novel strategy for pest management employs genotypic variant selection, mixing, coinfection, and intraspecific co-occlusion to generate highly harmful viral preparations. This method can be deployed over a broad variety of NPV-pest pathosystems because the narrow host range inhibits the absorption of these pesticides (26).

Fungal Biocontrol

Arthropod-harming fungi are common in tropical areas and play a key part in the balance of insect populations. The diversity of fungal spore variants, along with the different irregularities they elicit in the hosts they infect, combined, enhances the potency and incidence of infection (27). Entomopathogenic fungi such as *Beauveria bassiana* and *Trichoderma harzianum* are believed to be natural microbial control agents against various insects, including *Culex pipiens*, aiding in epidemic reduction. They start an infection in the gut, with fungal enzymes aiding in cuticle penetration. Toxin synthesis triggers the host's immune response, leading to host mortality. The host-fungus interaction is influenced by the epicuticle's structure and chemical content, with toxicity discrepancies among selected fungus isolates (28).

Macroorganisms

Algae (e.g., *G. elongata*, *J. rubens*, *C. tomentosum*, and *U. intestinalis*), Plants (e.g., *Teucrium polium*, *Peganum harmala*, *Thymus vulgaris* and *Physalis angulate*), Predators: larvivorous fish, ladybugs and dragonflies, mosquitofish, birds, and toads eat dangerous insects, including mosquitoes.

Algal-Based Biocontrol

Micro and macroalgae extracts show potential for mosquito control, however, some create hazardous chemicals. These extracts can impede feeding, gut membrane cells, digesting, and detoxifying enzymes, all of which affect the neurological system of larvae. Additional study is required to discover active molecules and assess

safety and effectiveness (29). Both red and green algae extracts were more hazardous to *Culex pipiens* larvae than brown algae extracts. As a result, *G. elongata*, *J. rubens* (Rhodophyta), *C. tomentosum*, and *U. intestinalis* (Chlorophyta) had stronger larvicidal activity than *P. boryana*, *D. dichotoma*, and *S. dentifolium* (Phaeophyta). However, *G. latifolium* (Red algae, Rhodophyta) is less potent than other red algae (30).

Fish-Based Biocontrol

Local fish genera such as *Danio rerio*, *Danio malabaricus*, and *Aplocheilus panchax* have substantial bio-control capability for mosquitoes, but more research is needed to understand their influence on aquatic ecosystems and biodiversity (31). A recent study shows that *B. splendens* and *A. panchax*, two surviving species, exhibit exceptional aggressiveness in various conditions, with *B. splendens* being most effective at low temperatures and *A. panchax*

most effective in hot lowland and high temperatures (32).

Plant-Based Biocontrol

Our ancestors may have relied on using plants to address the problem of mosquitoes. Extracts of plants such as *Teucrium polium*, *Peganum harmala*, *Thymus vulgaris* and *Physalis angulata* have been used, and their effects on *Culex pipiens* have been observed. The use of extracts of these plants harms mosquitoes and a positive effect on the environment, as they are less toxic substances and have their mechanical effect on the intestinal cells of the target insect. Detachment of cells and lining membranes and erosion of microvilli are all effects that ultimately lead to the elimination of the insect (33).

Table 1 shows several organisms that have been used in recent studies to test their ability to kill *Culex* mosquito larvae at different concentrations and larval numbers.

Table 1: Organisms' capacity to kill *Culex* mosquito larvae at different concentrations and larval numbers

Types of biocontrol	Organisms	Effect	Larval kill rate	Reference
Bacteria	<i>Bti</i>	Larvicidal	100%	(34)
	<i>Wolbachia</i>	Reduced fertility/ reproduction	17-28%	(35)
Fungi	<i>Beauveria bassiana</i>	Larvicidal	22%	(28)
	<i>Trichoderma harizanium</i>	Larvicidal	17.8%	(28)
Algae	<i>Jania rubens</i>	Larvicidal	96.67%	(36)
	<i>Colpomenia sinuosa</i>	Larvicidal	70%	(36)
Larvivorous fish	<i>Danio rerio</i>	Larvicidal feeds on larvae	62%	(31)
	<i>Danio malabaricus</i>	Larvicidal feeds on larvae	30%	(31)
	<i>Aplocheilus panchax</i>	Larvicidal feeds on larvae	58%	(31)
Plants	<i>Murraya koenigii</i> L.	Larvicidal	10%	(37)
	<i>Syzygium aromaticum</i>	Larvicidal	23.33%	(37)
	<i>Murraya koenigii</i> L.	Larvicidal	63.33%	(37)

The Role of BTI in *Culex pipiens* Control

Bacillus thuringiensis (Bt) is widely distributed throughout ecosystems. Nowadays, tens of thousands of *Bt* genotypes have been identified, discovered and secured globally, obtained from various environments such as soil (38). The facultative aerobic, Gram-positive, spore-forming saprophyte soil microorganism is a prominent ecological insecticide, representing 95% of commercial bioinsecticides, attributed to its particularity, reliability, and durability in managing many pest invasions (39). The ranking of Bt crystal toxins includes 73 families and six types of endotoxin proteins (40). Toxins, including δ -endotoxins, exotoxins, haemolysis, enterotoxins, and VIPs, are proteins or chemicals produced or stored in cells. Pest control mostly relies on δ -endotoxins composed of Cyt and Cry proteins. Insect larvae consume these toxic substances, which alter osmotic pressure, resulting in cell lysis and insect death (41).

Pore-forming toxins (PFT) are common virulence factors among bacterial pathogens, including human pathogens (42, 43). In insects, PFT disrupts host cell membranes by forming lytic pores but may also cause other effects beyond pore formation in various insect bacterial pathogens (44, 45). *BTi* proteins are toxic when ingested, targeting the host midgut epithelium. They are released as crystalline inclusions and activated by midgut proteases. These proteins bind specific receptors on the epithelial membrane, forming pores (46, 47). Once inserted, the membrane is disrupted, allowing gut bacteria to invade, leading to septicaemia and insect death (48). *Bti* bacteria depend on the presence of specific receptors within the host, so they target the specific type to kill it, while some organisms used, such as plant extracts or oil

extracts, kill the targeted and non-target organisms. For example, *Wolbachia* bacteria, which are endosymbiotic, have been identified as a potential method to combat mosquito-borne diseases like dengue, Zika, and Chikungunya. *Wolbachia* manipulates host reproduction and blocks arbovirus transmission, with cytoplasmic incompatibility (CI) being the most common reproductive manipulation. Other manipulations include parthenogenesis, feminisation, or male killing in some hosts (22). But like any living organism, after the insect ingests the bacteria or their toxins, its body begins to activate resistance mechanisms. Since the way bacteria enter the insect's body is through ingestion, the first step the insect takes is to change its feeding behaviour. To avoid consuming these compounds, they have evolved aversive taste reactions (49). Initial findings suggested that larvae treated with either *B. thuringiensis* proteins or a combination of spores and crystals altered their behaviour by ceasing to eat or becoming less active than nontreated larvae (50, 51). And this is just the beginning of many strategies. However, using *Bti* as a vector management not only minimises the threat of mosquitoes and the diseases they transmit, but also demonstrates that these bacteria can eliminate some of the pollutants in their surroundings (52). Several studies indicate that most strains of such bacteria tend to remove environmental pollutants, whether from water or soil. These pollutants include organic pollutants, petroleum hydrocarbons, pentachlorophenol, hexavalent chromium-contaminated wastewater, and organophosphorus pesticides (53, 54). *Bti* is a frequently administered pesticide for eliminating mosquitoes of *Culex pipiens*. The extensive reliance on these larvicides raises serious concerns regarding the development of resistance. The development and longevity of pesticide resistance have been related to fitness costs that may appear in difficult environments, such as during the winter (55). Insecticide resistance has been reported in *Culex pipiens* from Southern Europe and several countries neighbouring Europe (56). The use of *Bacillus* biocide in Europe has led to resistance in mosquitoes, with two forms, *pipiens* and *molestus*, exhibiting different overwintering biology. The dependency on Diflubenzuron (DFB) and *Bacillus thuringiensis* var. *israelensis*

(*Bti*) poses concerns for resistance development. A study found that short-term selection of both larvicides significantly reduced winter survival rates of *molestus* but not *pipiens*, suggesting potential differences in individual persistence (55). In a published scientific study, researchers examined the possibility of combining *Bti* with *L. sphaericus*. Several advantages of combining were summarised: Increases effectiveness, helps prevent the development of resistance in mosquitoes and enhances the effectiveness of mosquito control programs (57). In recent study reveals that *Bti* significantly affects the microbiota and gene expression of *Culex pipiens pallens* larvae. The predominant bacteria shift from Actinobacteria to Firmicutes, and *Bacillus* replaces *Microbacterium* in *Bti*-exposed groups. *Bti* infection also alters the diversity of microbiota, potentially inhibiting some bacteria and providing opportunities for other opportunistic taxa. Pathway analysis shows significant enhancements in sphingolipid metabolism, glutathione metabolism, and glycerophospholipid metabolism (58).

Conclusions

Using biological methods to limit the spread of mosquitoes that transmit diseases is a safe and effective approach. Although some vaccines are available for most diseases transmitted by mosquitoes, not all these pathogens have a vaccine available or are under development, so controlling mosquitoes is the ideal solution. Of course, eliminating chemical control methods that cause harm to the environment and non-target organisms is an ongoing goal. Biological control is feasible, especially by using strains of bacteria and even using genetic engineering to reduce the occurrence of resistance by mosquitoes. The best bacterial type is *Bacillus thuringiensis*. This is due to the availability of different toxins, and the property of this bacteria to specifically target the *Culex* mosquitoes has made it one of the best solutions. Since some studies have shown that *Bacillus thuringiensis* can decompose some pollutants, using it will be like hitting two birds with one stone. Although the total extermination of this type of mosquito may affect biodiversity and the food chain, therefore, consideration must be given in the future to develop genetically modified methods to target only the mosquitoes that carry the pathogen and not every individual mosquito. So, from here we can estimate an organism with the optimal ability that performs its work in a specialised way, targeting the insect itself without affecting other living organisms. Not only that, but we can say

that two birds have been killed with one stone, as by using *BTi* bacteria and replacing environmentally harmful pesticides, we avoid wasting time and effort in the future to treat the environment polluted with chemical pesticides

List of abbreviations

BTI: *Bacillus thuringiensis israelensis*
CHIKV: Chikungunya virus
EPNs: Entomopathogenic parasites
NPV: Nucleopolyhedroviruses
Bt: *Bacillus thuringiensis*

Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of data and materials

The data and materials associated with this research will be made available by the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

ANY conducted the study, including the design, data collection, analysis, and manuscript writing. The supervisors, UJN and JSIA, provided critical review, academic guidance, and valuable feedback throughout the research process.

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